

WHAT IS CLAIMED IS:

1. A method for producing a transgenic plant, comprising (a) agitating a solution, which comprises (1) a germinating plant seedling, or explant thereof, and (2) at least one *Agrobacterium* strain that comprises a vector, which comprises a desired polynucleotide; (b) cultivating the seedling to produce a plant; and (c) screening the plant to determine if the desired polynucleotide is integrated into the genome of at least one cell of the plant to produce a stably transformed plant, wherein the step of agitating the solution does not comprise sonication, and wherein the germinating plant seedling is exposed to an agent that enhances transformation efficiency before, during, or after the step of agitating the solution.
2. The method of claim 1, wherein the agent that enhances transformation efficiency is at least one of a purine inhibitor, a pyrimidine inhibitor, or a purine- and a pyrimidine- inhibitor.
3. The method of claim 1, wherein the agent is selected from the group consisting of mizoribine, azathioprine, mycophenolic acid, mycophenolate mofetil, 5-fluorouracil, Brequinar sodium, leflunomide, azaserine, acivicin, methotrexate, methotrexate polyglutamate derivatives, and cyclophosphamide.
4. The method of claim 2, wherein the agent is a purine inhibitor and a pyrimidine inhibitor.
5. The method of claim 4, wherein the agent is azaserine or acivicin.
6. The method of claim 1, wherein the agent induces chromosome breakage.
7. The method of claim 6, wherein the agent is methyl methane sulfonate.

8. The method of claim 1, wherein the vector comprises (a) a T-DNA or a P-DNA, which comprises (i) the desired polynucleotide, and (ii) a selectable marker gene operably linked to a terminator that is not naturally expressed in plants; and (b) a backbone integration marker gene, wherein the desired polynucleotide and the selectable marker gene are positioned between the border sequences of the T-DNA or between the border-like sequences of the P-DNA, and wherein the backbone integration marker gene is not positioned within the T-DNA or within the P-DNA.

9. The method according to claim 8, further comprising (i) producing a callus from the cultivated seedling; and (ii) inducing shoot and root formation from the callus, prior to transferring to soil to produce the plant.

10. The method of claim 9, wherein the step of producing the callus from the transformed seedling comprises (i) transferring the seedling that had been subjected to agitation to tissue culture media, which contains auxin and cyanamide; (ii) selecting a fertilizer-resistant callus; (iii) inducing shoot and root formation from the callus; and (iv) transferring a callus with shoots and roots to soil and exposing the callus to conditions that promote growth of a transgenic plant from the callus.

11. The method of claim 10, wherein expression of the selectable marker gene confers fertilizer resistance or cyanamide resistance to the transgenic plant and to progeny of the transgenic plant

12. The method of claim 11, wherein the selectable marker gene is a cyanamide resistance gene.

13. The method of claim 12, wherein the cyanamide resistance gene comprises the nucleotide sequence depicted in any one of SEQ ID NO. 12 or a variant thereof, SEQ ID NO. 14 or a variant thereof, or SEQ ID NO. 15 or a variant thereof, and wherein the gene encodes a protein that confers cyanamide resistance.

14. The method of claim 13, wherein the protein that confers cyanamide resistance comprises the sequence of SEQ ID NO. 13 or a variant thereof, wherein the variant protein is functionally active.

15. The method of claim 13, wherein the germinating plant seedling or explant thereof is a monocotyledonous plant and the cyanamide resistance gene (i) comprises the sequence of SEQ ID NO. 14, or a variant thereof, and (ii) encodes a functional cyanamide resistance protein.

16. The method of claim 13, wherein the plant seedling or explant thereof is a dicotyledonous plant and the cyanamide resistance gene (i) comprises the sequence of SEQ ID NO. 15, or a variant thereof, and (ii) encodes a functional cyanamide resistance protein.

17. The method of claim 1, wherein the germinating plant seedling is from a monocotyledonous plant.

18. The method of claim 17, wherein the monocotyledonous plant is selected from the group consisting of bentgrass, bluegrass, turfgrass, wheat, maize, rice, oat, barley, orchid, iris, lily, onion, sugarcane, and sorghum.

19. The method of claim 9, wherein the turfgrass is selected from the group consisting of *Agrostis spp.*, *Poa pratensis*, *Lolium spp.*, *Festuca arundinacea*, *Festuca rubra commutata*, *Cynodon dactylon*, *Pennisetum clandestinum*, *Stenotaphrum secundatum*, *Zoysia japonica*, and *Dichondra micrantha*.

20. The method of claim 1, wherein the germinating plant seedling is from a dicotyledonous plant.

21. The method of claim 20, wherein the dicotyledonous plant is selected from the group consisting of cotton, tobacco, *Arabidopsis*, tomato, potato, sugar beet, broccoli, cassava, sweet potato, pepper, poinsettia,

legumes, alfalfa, soybean, carrot, strawberry, lettuce, oak, maple, walnut, rose, mint, squash, daisy, geranium, and cactus.

22. The method of claim 1, wherein expression of the desired polynucleotide in the stably transformed plant confers a trait to the plant selected from the group consisting of increased drought tolerance, reduced height, enhanced cold and frost tolerance, improved vigor, enhanced color, enhanced health and nutritional characteristics, improved storage, enhanced yield, enhanced salt tolerance, enhanced heavy metal tolerance, increased disease tolerance, increased insect tolerance, increased water-stress tolerance, enhanced sweetness, improved taste, improved texture, decreased phosphate content, increased germination, increased micronutrient uptake, improved starch composition, improved flower longevity, and production of novel proteins or peptides.

23. The method of claim 1, wherein the desired polynucleotide expresses a peptide or protein that is an antifungal, a nutritional peptide or protein, a transcription factor, a receptor that binds to pathogen-derived ligands, a hemoglobin, an oxidase, an enzyme of the lignin biosynthesis pathway, an enzyme of industrial value, or an antigen.

24. The method of claim 8, wherein the backbone integration marker is a cytokinin gene.

25. The method of claim 24, wherein the cytokinin gene is *IPT*, and the plant is a dicotyledonous plant.

26. The method of claim 8, wherein the backbone integration marker is *PGA22*, *TZS*, *HOC1*, *CKI1*, and *ESR1*.

27. The method of claim 1, wherein the step of agitating the solution is accomplished by vortexing.

28. The method of claim 27, wherein the solution is vortexed from about 60 seconds to several hours.

29. The method of claim 28, wherein the solution is vortexed for about 5 minutes to about 30 minutes.

30. The method of claim 1, wherein the step of cultivating the seedling to produce a transgenic plant comprises transferring the *Agrobacterium*-transformed seedling to soil, and exposing the transformed seedling to conditions that promote growth.

31. The method of claim 1, wherein the step of cultivating the seedling to produce a transgenic plant comprises cultivating the *Agrobacterium*-transformed seedling in or on tissue culture medium prior to transferring the transformed seedling to soil, and exposing the transformed seedling to conditions that promote growth.

32. The method of claim 1, wherein the transformed plant seedling is grown to maturity, crossed to a non-transformed plant and the desired polynucleotide transmitted to at least one progeny plant.

33. The method of claim 1, wherein the transformed plant seedling is grown to maturity, selfed, and the desired polynucleotide transmitted to progeny.

34. A method for producing a transgenic plant, comprising (a) agitating a solution that comprises (1) a germinating plant seedling and (2) at least one *Agrobacterium* strain that comprises a vector, which comprises (i) a desired polynucleotide and (ii) a cyanamide resistance gene; (b) (i) producing a callus from the transformed seedling and (ii) inducing shoot and root formation from the callus to produce plantlets; (c) growing the plantlets into plants; and (d) screening the plants to determine if the desired polynucleotide is incorporated into the genome of at least one cell of the plant

to produce a stably transformed transgenic plant, and wherein the step of agitating the solution does not comprise sonication.

35. The method of claim 34, wherein the desired polynucleotide and the cyanamide resistance gene, which is operably linked to a terminator that is not naturally expressed in plants, are positioned between border or border-like sequences of a T-DNA or a P-DNA located in the vector.

36. The method of claim 35, wherein the cyanamide resistance gene comprises the nucleotide sequence of any one of SEQ ID NOs. 12 or a variant thereof, SEQ ID NO. 14 or a variant thereof, or SEQ ID NO. 15 or a variant thereof, and wherein the cyanamide resistance gene encodes a protein that comprises the amino acid sequence of SEQ ID NO. 13.

37. The method of claim 36, wherein the vector further comprises a backbone integration marker gene, which is not positioned between the border or border-like sequences of the T-DNA or the P-DNA.

38. The method of claim 34, further comprising exposing the germinating plant seedling to an agent that enhances transformation efficiency.

39. The method of claim 38, wherein the agent that enhances transformation efficiency is at least one of a purine inhibitor, a pyrimidine inhibitor, or a purine- and a pyrimidine- inhibitor.

40. The method of claim 39, wherein the agent is selected from the group consisting of mizoribine, azathioprine, mycophenolic acid, mycophenolate mofetil, 5-fluorouracil, Brequinar sodium, Leflunomide, azaserine, acivicin, methotrexate, methotrexate polyglutamate derivatives, and cyclophosphamide.

41. The method of claim 34, wherein the agent induces chromosomal breakage.

42. The method of claim 41, wherein the agent is methyl methane sulfonate.

43. The method of claim 34, wherein the step of agitating the solution is accomplished by vortexing.

44. The method of claim 43, wherein the solution is vortexed from about 60 seconds to several hours.

45. The method of claim 44, wherein the solution is vortexed for about 5 minutes to about 30 minutes.

46. An isolated nucleic acid comprising the sequence of SEQ ID NO. 12, or variant thereof, wherein the nucleic acid encodes a functional cyanamide resistance protein.

47. The isolated nucleic acid of claim 46, wherein the cyanamide resistance protein comprises the amino acid sequence of SEQ ID NO. 13 or variant thereof.

48. An isolated nucleic acid comprising the sequence of SEQ ID NO. 14, or variant thereof, wherein the nucleic acid encodes a functional cyanamide resistance protein.

49. The isolated nucleic acid of claim 48, wherein the cyanamide resistance protein comprises the amino acid sequence of SEQ ID NO. 13 or variant thereof.

50. An isolated nucleic acid comprising the sequence of SEQ ID NO. 15, or variant thereof, wherein the nucleic acid encodes a functional cyanamide resistance protein.

51. The isolated nucleic acid of claim 50, wherein the cyanamide resistance protein comprises the amino acid sequence of SEQ ID NO. 13 or variant thereof.

52. An isolated cyanamide resistance protein comprising the amino acid sequence of SEQ ID NO. 13, or variants thereof, wherein the protein confers resistance to cyanamide.

53. The isolated cyanamide resistance protein of claim 52, wherein the variant has a sequence identity of at least 80% to the amino acid sequence of SEQ ID NO. 13.